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Dicing up chromosomes

The unexpected role of dicer in apoptosis

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Apoptosis is an evolutionarily conserved and tightly reglated process in which caspases, a unique family of cysteine proteases, direct the dismantling of a dying cell. A fundamental feature of apoptosis is chromosome fragmentation, which is executed by multiple apoptotic nucleases in a stepwise manner.[1] Apoptotic nucleases first introduce 3' hydroxyl nicks into chromosomes, which are detectable by terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL). The 3' hydroxyl DNA nicks are then converted into single-stranded gaps via exonuclease activity and double-stranded breaks via gap-dependent endonuclease activity,[1] losing their TUNEL-reactive ends in the process.

Generation of 3' hydroxyl nicks in mammals is performed by 40-kD DNA fragmentation factor (DFF40). In living cells, DFF40 complexes with 45-kD DNA fragmentation factor (DFF45), a cognate inhibitor of DFF40. In dying cells, activated caspase-3 and caspase-7 cleave DFF45 to release DFF40, which initiates chromosome fragmentation by generating TUNEL-reactive DNA ends.[2] Homologues of DFF40 or DFF45 have not been identified in the *C. elegans* genome. However, apoptotic factors important for resolution of TUNEL-reactive DNA ends appear to be conserved between mammals and nematodes. For example, endonuclease G (EndoG) and apoptosis-inducing factor (AIF) and their corresponding *C. elegans* homologues CPS-6 (CED-3 protease suppressor 6) and WAH-1 (worm AIF homologue) translocate from mitochondria to the nucleus to facilitate chromosome fragmentation and cell death.[3-5] DNase II and its *C. elegans* homologues, NUC-1 (abnormal nuclease) and CRN-6 (cell death-related nuclease 6), then conclude the DNA degradation process by completely digesting fragmented chromosomes (Figure).

In *C. elegans* activation of the single cell-killing caspase CED-3 precedes the action of at least nine apoptotic nucleases – CPS-6, NUC-1, and seven cell death-related nucleases (CRNs).[1] Deficiency in any of these nucleases results in accumulation of TUNEL-stained nuclei, suggesting that all nine nucleases are involved in resolving TUNEL-reactive DNA ends. With the exception of NUC-1 and CRN-6, inactivation of these nuclease genes also results in delayed developmental cell death. However, the mechanism of initial DNA cleavage during apoptosis in *C. elegans* remained enigmatic until recently, when we observed that the *C. elegans* Dicer (DCR-1) ribonuclease is cleaved by the activated CED-3 caspase into a truncated C-terminal fragment (tDCR-1) that exhibits DNase activity, makes initial TUNEL-reactive DNA nicks, and promotes apoptosis upstream of the previously characterized nucleases.[6]

DCR-1 contains a helicase domain, a PAZ (Piwi, Argonaute, Zwille) domain, two RNase III domains, and a double-stranded RNA (dsRNA)-binding domain.[7] The two RNase III domains, RNase IIIa and IIIb, reside in the C-terminus of DCR-1 and process precursor dsRNA molecules into small RNAs, such as microRNAs and small interfering RNAs, which regulate gene expression during development.[8] Interestingly, in cells undergoing apoptosis, CED-3 cleaves DCR-1 within the RNase IIIa domain to generate tDCR-1, which still contains an intact RNase IIIb domain. tDCR-1 lacks RNase activity but possesses a new DNase activity that produces 3' hydroxyl DNA nicks and initiates apoptotic chromosome fragmentation in *C. elegans*. Thus, despite sharing no sequence similarity, DCR-1/tDCR-1 are functional analogues of mammalian DFF45/DFF40 and undergo a conserved caspase-mediated mechanism to activate chromosome fragmentation during apoptosis (Figure).

This finding is surprising for several reasons. This is the first example that one type of enzyme (a RNase) is proteolytically converted into a different type of enzyme (a DNase) with different substrate specificity. Second, the dicer nucleases have been thought to be exclusively involved in processing and generating small RNAs. The discovery that DCR-1 can be converted into a DNase through proteolytic processing raises the possibility that some dicer functions could be mediated by its DNase activity. Third, the *dcr-1* gene had not previously been identified as a direct regulator of apoptosis. Given the similarity in sequence and function among dicer nucleases in different species, our finding suggests that dicer could directly affect apoptosis in other organisms.

Important questions remain regarding this unexpected finding. For example, it will be interesting to determine whether mammalian dicer nucleases can acquire DNase activity and promote apoptosis like *C. elegans* dicer, as there are reports that human dicer is also cleaved by caspases during apoptosis.[9,10] In addition, the mechanistic basis of DCR-1's RNA-to-DNA substrate switch following cleavage by CED-3 is not understood and demands further biochemical and structural

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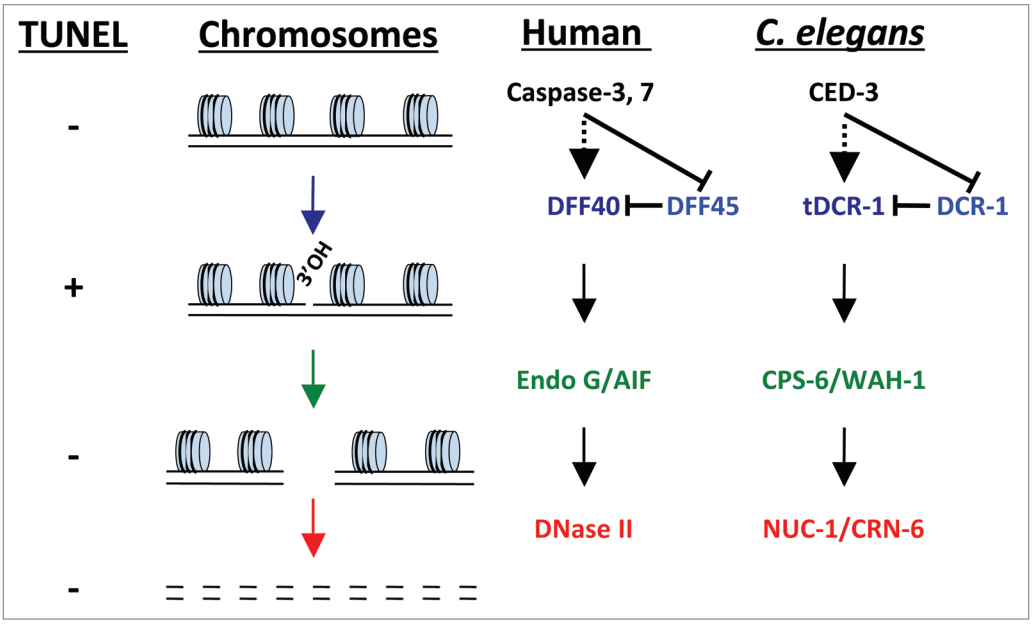

Figure 1. Pathways for chromosomal fragmentation during apoptosis are conserved between humans and *C. elegans*. In healthy human cells, the initiator nuclease DFF40 is inhibited by DFF45. Similarly, in living *C. elegans* cells, DCR-1 masks the activity of tDCR-1. Upon apoptosis activation, caspase-3 and caspase-7 in humans and CED-3 in *C. elegans* cleave their respective substrates, DFF45 and DCR-1, to activate DFF40 and tDCR-1, which introduce 3′ hydroxyl nicks into chromosomes and render them reactive to the TUNEL assay. TUNEL reactivity is lost as nicks are converted into double-stranded breaks by nuclease complexes containing Endo G/AIF in humans and CPS-6/WAH-1 in *C. elegans*. Finally, chromosomes are fully digested by DNase II nucleases.

characterization. This may not be the only example of conversion of a RNase into a DNase, or of a RNA-specific regulator into a DNA-specific regulator. Such a mechanism for substrate switching could be exploited to activate or alter a biological process in vivo, which could aid in development of new therapeutic strategies to treat human diseases.

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